Patent Claims

1. A method for the detection of cytosine methylation in DNA samples, is hereby characterized in that the following steps are conducted:

a) a genomic DNA sample is treated in such a way that the unmethylated cytosine bases are converted to uracil, while the 5-methylcytosine bases remain unchanged;

b) the chemically treated DNA sample is amplified with the use of at least 2 pairs of essentially complementary probe oligonucleotides as well as a ligase and

c) the amplificates are analyzed and the methylation status in the DNA to be investigated is concluded from the presence of an amplificate.

2. The method according to claim 1, further characterized in that in the second step, the DNA to be investigated is preferred over the sequence-homologous background DNA as the template.

3. The method according to one of claims 1 or 2, further characterized in that the methylation status in the DNA to be investigated is concluded from the analysis of additional positions in the amplificate.

4. The method according to one of the preceding claims, wherein in step b) of claim 1, the probe oligonucleotides then hybridize to a template, if the CpG positions which are covered by these in the genomic DNA sample (or the DNA to be investigated) were

methylated and wherein the same probe oligonucleotides hybridize essentially to a lesser extent to templates which were completely or partially unmethylated at these positions,.

5. The method according to one of claims 1 to 3, wherein in step b) of claim 1, the probe oligonucleotides then hybridize to a template, if the CpG positions which are covered by these in the genomic DNA sample (or the DNA to be investigated) were unmethylated and wherein the same probe oligonucleotides hybridize essentially to a lesser extent to templates which were present completely or partially methylated at these positions,.

6. The method according to one of the preceding claims, further characterized in that step b) of claim 1 is designed in detail as follows:

a) the probe oligonucleotides, which hybridized to adjacent positions on the template are coupled together by ligation,

b) the coupled probe oligonucleotides are dehybridized,

c) probe oligonucleotides complementary to the coupled probe oligonucleotides hybridize to the already coupled probe oligonucleotides and are coupled in turn by ligation and

d) the coupled probe oligonucleotides serve as a template for further ligation steps, so that a further propagation of the coupled probe oligonucleotides is produced.

7. The method according to one of the preceding claims, further characterized in that at least one of the probe oligonucleotides bears a phosphate group at the 5'-end.

8. The method according to one of the preceding claims, further characterized in that at least one of the probe oligonucleotides is provided with a label detectable by fluorescence.

9. The method according to one of the preceding claims, further characterized in that at least one of the probe oligonucleotides is provided with a detectable label.

10. The method according to claim 8 or 9, further characterized in that at least two probe oligonucleotides are provided with labels, and that these modify their properties as a function of the distance between them.

11. The method according to one of claims 8 to 10, further characterized in that the probe oligonucleotides bear at least one fluorescent label.

12. The method according to one of claims 8 to 11, further characterized in that the probe molecules indicate the amplification either by an increase or a decrease of the fluorescence.

13. The method according to claim 12, further characterized in that the increase or decrease in fluorescence also is used directly for the analysis and a conclusion on the methylation status of the DNA to be investigated is made from the modified fluorescent signal.

14. The method according to one of the preceding claims, further characterized in that the background DNA is present in 100x the concentration in comparison to the DNA to be investigated.

15. The method according to one of the preceding claims, further characterized in that the background DNA is present in 1000x the concentration in comparison to the DNA to be investigated.

16. The method according to one of the preceding claims, further characterized in that the DNA samples are obtained from serum or other body fluids of an individual.

17. The method according to one of the preceding claims, further characterized in that the DNA samples are obtained from cell lines, blood, sputum, stool, urine, serum, cerebrospinal fluid, tissue embedded in paraffin, for example, tissue from eyes, intestine, kidney, brain, heart, prostate, lungs, breast or liver, histological slides and all possible combinations thereof.

18. The method according to one of the preceding claims, further characterized in that step a) of claim 1 is conducted with a bisulfite (= disulfite, hydrogen sulfite).

19. The method according to claim 18, further characterized in that the chemical treatment is conducted after embedding the DNA in agarose.

20. The method according to claim 18, further characterized in that a reagent that denatures the DNA duplex and/or a radical trap is (are) present in the chemical treatment.

21. The method according to one of the preceding claims, further characterized in that the analysis according to claim 1c) is made by means of hybridization to oligomer arrays, wherein oligomers can be nucleic acids or molecules such as PNAs that are similar in their hybridization properties.

22. The method according to one of the preceding claims, further characterized in that the analysis according to claim 1c) is made by means of measuring the length of the amplified DNA to be investigated, whereby methods for length measurement comprise gel electrophoresis, capillary gel electrophoresis, chromatography (e.g. HPLC), mass spectrometry and other suitable methods.

23. The method according to one of the preceding claims, further characterized in that the analysis according to claim 1c) is conducted by means of sequencing, whereby methods for sequencing comprise the Sanger method, the Maxam-Gilbert method, and other methods such as sequencing by hybridization (SBH).

24. The method according to one of the preceding claims, further characterized in that a conclusion is made on the presence of a disease or another medical condition of the patient from the methylation status at the different CpG positions investigated.

25. The method according to one of the preceding claims, further characterized in that the amplificates themselves are provided with a detectable label for the detection.

26. The method according to claim 25, further characterized in that the labels are fluorescent labels.

27. The method according to claim 25, further characterized in that the labels are radionuclides.

28. The method according to claim 25, further characterized in that the labels are removable mass labels which are detected in a mass spectrometer.

29. The method according to claim 25, further characterized in that the amplificates are detected as a whole in the mass spectrometer and are thus clearly characterized by their mass.

30. The method according to one of the preceding claims, further characterized in that in addition to the probe oligonucleotides, a blocker oligonucleotide is utilized, which preferably binds to the background DNA and prevents the hybridization of the probe oligonucleotides to the background DNA.

31. The method according to claim 30, further characterized in that two blocker oligonucleotides (or blocker PNAs, generally blocker molecules) that are complementary

to one another are used.

32. The method according to claim 31, further characterized in that the blocker molecules preferably bind to template strands, which correspond in their sequence to a DNA that is methylated after treatment according to claim 1a).

33. The method according to claim 31, further characterized in that the blocker molecules preferably bind to template strands, which correspond in their sequence to a DNA that is unmethylated after treatment according to claim 1a).

34. The method according to one of claims 31 to 33, further characterized in that the blocker molecules bind to several CpG positions in the template DNA.

35. The method according to one of claims 31 to 33, further characterized in that the blocker molecules bind to several TpG or CpA positions in the template DNA.

36. The method according to one of claims 31 to 35, further characterized in that the blocker oligonucleotides are modified at their 3'-end and cannot be essentially decomposed by a polymerase with nuclease activity.

37. The method according to one of the preceding claims, further characterized in that step b) of claim 1 is designed in detail as follows:

a) the probe oligonucleotides (probes) hybridize to positions on the template

strand in such a way that a gap of at least one base remains between the 3'-end of the first probe and the 5'-end of the second probe,

b) the 3'-end of the first probe is extended by a polymerase reaction, wherein nucleotides complementary to the template strand are incorporated each time,

c) the elongated first probe is coupled by ligation to the elongated second probe,

d) the coupled probe oligonucleotides are dehybridized,

e) probe oligonucleotides complementary to the coupled probe oligonucleotides hybridize to the already coupled probe oligonucleotides and are coupled in turn by ligation and

f) the coupled probe oligonucleotides serve as a template for further ligation steps, so that a further propagation of the coupled probe oligonucleotides is produced.

38. The method according to claim 37, further characterized in that step e) of claim 37 is also conducted analogously to steps a) - c).

39. The method according to one of claims 37 or 38, further characterized in that a heat-stable polymerase is used.

40. The method according to one of the preceding claims, further characterized in that a heat-stable ligase is used.

41. The method according to one of the preceding claims, further characterized in that several sets of oligonucleotide probes are utilized for several groups of methylation

positions and thus a multiplexing of the assay is achieved.

42. Use of a method according to one of the preceding claims for the diagnosis and/or prognosis of adverse events for patients or individuals, whereby these adverse events belong to at least one of the following categories: undesired drug interactions; cancer diseases; CNS malfunctions, damage or disease; symptoms of aggression or behavioral disturbances; clinical, psychological and social consequences of brain damage; psychotic disturbances and personality disorders; dementia and/or associated syndromes; cardiovascular disease, malfunction and damage; malfunction, damage or disease of the gastrointestinal tract; malfunction, damage or disease of the respiratory system; lesion, inflammation, infection, immunity and/or convalescence; malfunction, damage or disease of the body as a consequence of an abnormality in the development process; malfunction, damage or disease of the skin, the muscles, the connective tissue or the bones; endocrine and metabolic malfunction, damage or disease; headaches or sexual malfunction.

43. Use of a method according to one of the preceding claims for the differentiation of cell types or tissues or for the investigation of cell differentiation.

44. A kit comprising a reagent containing bisulfite, labeled oligonucleotide probes, a preferably heat-stable ligase and buffers, as well as, optionally, instructions for conducting an assay according to the invention.